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THE ETIOLOGY OF COMMON COLDS *

THE PROBABLE ROLE OF A FILTRABLE VIRUS AS THE CAUSATIVE FACTOR: WITH EXPERIMENTS ON THE CULTIVATION OF A MINUTE MICRO-ORGANISM FROM THE NASAL SECRETION FILTRATES

PLATE 9

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Numerous attempts have been made to demonstrate the specific cause of the extremely prevalent catarrhal affections of the upper respiratory tract, popularly known as "common colds." In reviewing the literature, however, one is struck by the multiplicity of organisms to which an etiologic relation has been ascribed. *B. influenzae*, *Strep. hemolyticus*, *Strep. viridans*, the pneumococcus, *M. catarrhalis*, *B. septus*, Friedlander's bacillus and Tunnicliff's *B. rhinitis* have each held the stage. Some investigators believe that micro-organisms bear no relation whatever to the causation of colds; but that many factors—wet and cold, drafts, irritating vapors, overheated rooms, worry, fatigue, sexual excesses, dietetic errors, alcohol, and what not—enter into the etiology. Still another group takes the stand that a number of conditions—notably chilling, sudden changes in temperature, and fatigue—act as predisposing factors by lowering resistance, and thus pave the way for infection by micro-organisms commonly believed to be present normally in the nasal cavities.

In short, present knowledge of the etiology of the common cold is in a most chaotic state. As remarked by Stitt,¹ "Of all the diseases common in man this condition has been surrounded by greater etiological and epidemiological obscurity than any other."

CLINICAL DEFINITION OF THE "COMMON COLD"

Much confusion in the interpretation of the reported findings of various investigators has resulted from classifying various respiratory infections under one heading. It is an almost universal custom to

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¹ Practical Bacteriology, Ed. 4, 1916, p. 435.

classify as common colds mild and transitory rhinorrheas, without systemic manifestations and due to vasomotor disturbances; the ordinary acute coryza, with its slight or moderate systemic disturbances; and the so-called grip or influenza, characterized by more or less marked systemic reaction and prostration.

The cases studied in this investigation conform to the entity designated by Osler² "acute catarrhal fever (acute coryza)," the symptoms of which he describes as follows:

The patient feels indisposed, perhaps chilly, has slight headache, and sneezes frequently. In severe cases there are pains in the back and limbs. There is usually slight fever, the temperature rising to 101 F. The pulse is quick, the skin is dry, and there are all the features of a feverish attack. At first the mucous membrane of the nose is swollen, "stuffed up," and the patient has to breathe through the mouth. A thin, clear, irritating secretion flows, and makes the edges of the nostrils sore. The mucous membrane of the tear ducts is swollen, so that the eyes weep and the conjunctivae are injected. The sense of smell and, in part, the sense of taste are lost. With the nasal catarrh there is slight soreness of the throat and stiffness of the neck; the pharynx looks red and swollen, and sometimes the act of swallowing is painful. The larynx may also be involved and the voice becomes husky or is even lost. If the inflammation extends to the eustachian tubes the hearing may be impaired. In more severe cases there are bronchial irritation and cough. Occasionally there is an outbreak of labial or nasal herpes. Usually within thirty-six hours the nasal secretion becomes turbid and more profuse, the swelling of the mucosa subsides, the patient gradually becomes able to breathe through the nostrils, and within four or five days the symptoms disappear, with the exception of the increased discharge from the nose and upper pharynx. There are rarely any bad effects from a simple coryza. When the attacks are frequently repeated the disease may become chronic.

PRELIMINARY BACTERIOLOGIC STUDIES

Selecting cases that conformed to the type just described—usually within 24 hours of the onset—several platinum loopfuls of nasal secretion were emulsified in sterile salt solution, and varying amounts of this emulsion were used in the preparation of poured human blood-agar plates. One series of plates was incubated aerobically at 37 C., and a duplicate series was similarly incubated under strictly anaerobic conditions. The secretions from 16 cases were examined in this way.

As a rule, the plates prepared from secretions collected within 24 hours of the onset, while the nasal secretion was thin and clear, remained sterile or showed, at most, but few colonies, the identity of which varied in different cases. Many writers note this paucity of bacteria in the nasal secretion from early coryzas. On the other hand, secretions that had become mucopurulent or blood-streaked uniformly

² The Principles and Practice of Medicine, Ed. 8, 1916, p. 382.

gave innumerable colonies in plate cultures, but here again there was no uniformity in the findings. In these cases various hemolytic streptococci, *Strep. viridans*, *M. catarrhalis*, and the pneumococcus predominated in numbers in the order named.

I desire to lay emphasis on the negative findings in early cases as challenging the popular belief that the nasal cavities furnish a normal habitat for various bacteria capable of pathogenicity under certain conditions. Numerous workers have shown that the normal nasal mucosa is sterile or practically so. Cecil,³ in an investigation of *Strep. viridans* in its relation to infections of the upper respiratory tract, found that cultures from coryzas in the acute stage were often sterile, and he states that cultures taken in the subacute or chronic stages often show so many different types that it is difficult to decide which organism is the causative factor. He further found that *Strep. viridans* infections nearly always start in the throat and extend upward or downward, or both.

It became apparent early that nothing tangible was to be gained by resorting further to the usual bacteriologic methods in searching for a specific causative factor, and the problem was approached from another point of view.

DEMONSTRATION OF A FILTRABLE VIRUS

Some time ago, Kruse⁴ pointed out that while common colds are undoubtedly infectious, the scarcity of bacteria in the secretions and the short time that these few persist militates against the purely presumptive evidence that they are concerned in the infection. In attempting to trace the causative factor by means other than the bacteriologic methods that had been resorted to previously, he succeeded in producing colds experimentally with a filtrate obtained from the nasal secretion of an assistant who was ill with coryza. The secretions blown from the nose were diluted 15 times with normal salt solution, and passed through a small Berkefeld filter. A few drops of the filtrate dropped on the nasal mucosa of each of 12 men produced acute colds in 4, or 33% of the 12. Repetition of this experiment on a more elaborate scale gave confirmation to his earlier results. In the second experiment the nasal secretion was diluted 20 times and, of the 36 students who volunteered for inoculation, 42% became ill with the usual symptoms of coryza within 1-4 days. Kruse states that he was unable to demonstrate living organisms in his filtrates by bacteriologic methods, and concludes that the causative organism should be classified with the filtrable viruses.

During the winter of 1915-1916 I succeeded in confirming Kruse's observations and this fact, together with results that suggested that a living virus had been cultivated in vitro, was communicated in a

³ The Laryngoscope, 1915, 25, No. 2, p. 97.

⁴ München. med. Wehnschr., 1914, 61, 1547.

preliminary note⁵ in April, 1916. In the present communication the experimental work briefly outlined in the preliminary report will be reviewed in detail and additional notes, on the cultivation of a minute filterable micro-organism from nasal secretion filtrates, reported.

MATERIAL

The first nasal secretion studied was obtained from one of the laboratory staff who had been ill with an acute coryza for two days. He complained of lassitude, chilly sensations, sneezing, unilateral nasal stuffiness, dull frontal headache with a feeling of oppression over the eyes, impairment of smell, and moderate aching pain in the extremities. There was the usual picture of an acute cold: injected conjunctivae, considerable lacrimation, a copious, thin, mucoid nasal discharge which excoriated the upper lip and the alae of the nose, and a very red, moist, swollen and boggy mucosa. The temperature was normal.

The second nasal secretion was collected on the first day of an acute cold experienced by myself. This attack was manifested by symptoms almost identical with those of the first case.

The third specimen was obtained from an employee of one of the large department stores who had been ill for three days. She complained of malaise, feverishness, aching pain in the back, nasal stuffiness, sneezing, frontal headache, dull pain over the eyes, impairment of taste and smell, slight soreness of the throat, and moderate cough with expectoration. The nasal mucosa was moist, red, swollen, and boggy. The nasal secretion was scant and mucoid, and showed admixture of shreds of purulent material. The nasal alae were markedly excoriated and the eyelids were red and puffy. This patient felt certain that she had had considerable fever the preceding evening, but the temperature was normal at the time the nasal secretion was collected.

THE PREPARATION OF FILTRATES

In each case the nasal secretion was blown from the nose into a sterile Petri dish, mixed with 10 c.c. of sterile 0.8% salt solution, and carried to the laboratory in a sterile test tube. The material was then poured into a sterile shaking bottle containing smooth round glass beads, and agitated for 10 minutes in a shaking machine. A thoroughly homogenous mixture, free from clumps or strings of mucus, resulted. This mixture was then passed through a small Berkefeld N filter. The filters used in this work were new, their reaction had been neutralized by boiling and repeated washing, and they were impermeable to ordinary bacteria. Filtration was accelerated by suction from a Chapman water pump attached to the laboratory tap. The resulting rate of filtration was such that about 5 c.c. of brilliantly clear filtrate was collected in 15 minutes. Human blood-agar plates prepared from the filtrates and incubated at 37 C., under both aerobic and anaerobic conditions, remained sterile at the end of 7 days.

INOCULATION TESTS WITH NASAL SECRETION FILTRATES

Ten soldiers at Fort Banks, Mass., who volunteered for the experiment, were inoculated with the filtrates described. The men were in good health at the time. In each case the nose was mechanically

⁵ Jour. Am. Med. Assn., 1916, 66, p. 1180.

cleansed with water, but no predisposing factors to infection, such as irritating or abrading the mucosa, were added. Inoculation was performed by simply tilting the head back so that the material would gravitate into the nose, and the filtrate was dropped into the nasal opening from a capillary pipet. From 3-6 drops of filtrate were placed in each nostril.

CASE 1.—The patient stated that he never remembered having had a cold. All members of his family—wife, sister, and 3 children—had repeated respiratory infections during the winter and, despite intimate daily contact, he remained well. This would indicate a probable high degree of immunity.

He was inoculated with 3 drops of nasal secretion Filtrate B in each nostril, at 9 a. m., February 22.

He was under observation for 4 days. No symptoms occurred.

CASE 2.—There was no history of coryza during the winter. The patient stated that he was not prone to colds. He recovered from an attack of acute tonsillitis a week prior to inoculation.

He was inoculated at 9 a. m., February 22, with 4 drops of nasal secretion Filtrate B in each nostril.

About 8 hours after inoculation his nose became dry and stuffy and by evening he was forced to breathe through his mouth. During the night he sneezed incessantly and "ringing in the ears" developed. The following morning his "head was stopped up," and he complained of slight cough. There was dull frontal headache, with pain over both orbits. The temperature and pulse were normal. When next seen, 48 hours after inoculation, the nasal mucosa was very red, swollen and boggy, and the left nostril was occluded, causing mouth breathing. The voice had a distinct nasal twang. The throat felt very dry, and there was complaint of pain over the frontal sinuses. The temperature was 99 F., and the pulse was 96. Treatment was started.

CASE 3.—There was no history of respiratory infections during the winter, no proneness to colds, and no history of recent exposure.

He was inoculated at 9 a. m., February 22, with 5 drops of nasal secretion filtrate in each nostril. Virus B was used.

The soldier stated that slight nasal discharge started 12 hours after inoculation. The following morning, 24 hours after inoculation, he complained of nasal stuffiness, and had several attacks of sneezing. About 4 p. m. in the afternoon he developed frontal headache, with dull pain over the left eye. The patient stated that he did not feel feverish. The following morning, 48 hours after inoculation, he complained of a slight unproductive cough. The nasal mucosa was dry, glazed, and tense, and there was no secretion. The headache had subsided, and the temperature was normal. Profuse rhinorrhea developed 4 hours later; the cough became worse, and headache returned. On the 4th day the nasal mucosa was still very red, swollen, and boggy, and it was bathed with mucoid secretion. The air space on the right side was occluded by the swollen mucosa. The nasal alae were excoriated. The temperature was 98 F. Except for discomfort occasioned by nasal stuffiness he felt well and on the 5th day all symptoms had ameliorated.

CASE 4.—The soldier was prone to colds. He was inoculated at 9 a. m., February 22, with 6 drops of nasal secretion Filtrate B in each nostril.

The onset of symptoms occurred about 30 hours after inoculation, with a most disagreeable sensation of fulness in back of the eyes. He stated that his nose was "as dry as a bone," and that his throat felt parched. During the night he got up repeatedly to quench his thirst, and flush out his nose. The nasal mucosa was so swollen that he was forced to breathe through his mouth, and he attributed the dryness of the throat to this. When seen again, 48 hours after inoculation, he felt listless and out of sorts, and complained of dull frontal headache, vertigo, and dryness of the nose and throat. The temperature was 99.4 F., and the pulse was 90. On the 3rd day the nasal mucosa was still dry and tense, but lassitude, sensation of fulness in the head, and nasal stuffiness were less troublesome. The lower lip was considerably swollen and covered with herpes. He stated that on arising in the morning he experienced a sharp stitch of pain in the lumbar region, while putting on his shoes. This pain disappeared after he had "limbered up" by walking around for a time. There was complaint of occasional sensations of flushing—"flashes of heat," as he expressed it. The temperature at this time was 99 F., and the pulse was 88. On the 4th day he felt entirely well.

CASE 5.—The patient had 2 attacks of coryza during the winter, the last a month prior to inoculation. He was not unduly prone to colds.

He was inoculated at 9 a. m., February 22, with 5 drops of nasal secretion Filtrate A in each nostril.

The soldier was sent on an ambulance call 24 hours after inoculation and, although the weather was moderate and he was warmly dressed, he had repeated sensations of chilliness during the trip. He developed profuse rhinorrhea 2 hours after returning to the hospital. During the night he complained of dull frontal headache, attacks of chilliness alternated with sensations of flushing, and the nasal alae and upper lip became excoriated from the copious, thin, watery nasal discharge. When seen again, 48 hours after inoculation, he complained of lassitude, "ringing in the ears," slight backache, a sensation of fulness over the frontal sinuses, and moderately dry, unproductive cough. The temperature was 99 F., and pulse was 80. On the 3rd day all symptoms had ameliorated, and the temperature was normal. He still felt languid and the frontal headache had not disappeared entirely. The nasal discharge was less copious and thicker. On the 4th day he complained of slight soreness of the throat and the frontal headache persisted. The temperature registered 99.4 F. The following day, 5 days after inoculation, his only complaint was of stiffness of the muscles of the neck and rheumatoid pains in the right arm. The 6th day he was quite himself again, except for occasional rheumatoid twinges in the muscles of the arm.

CASE 6.—The patient had several colds during the winter. The last attack was 3 weeks prior to inoculation. There was no history of recent exposure.

He was inoculated at 9 a. m., February 22, with 5 drops of nasal secretion Filtrate A in each nostril.

The soldier stated that slight frontal headache and nasal stuffiness developed about 6 hours after inoculation. He slept well and had no symptoms on awakening. About 2 p. m., February 23, he had several paroxysms of sneezing, and the throat felt dry and sore. During the evening moderate mucoid nasal discharge was noted, and headache returned. On the 3rd day the conjunctivae were injected, there was moderate lacrimation, the eyelids were red and puffy, the nasal mucosa was very red, swollen, and edematous, and the upper lip and alae of the nose showed the characteristic irritation from the nasal discharge. The tonsils were slightly swollen and edematous, and showed a few patches of filmy surface exudate. The entire pharynx was reddened. There was no

glandular enlargement, but the left tonsil was palpable externally and tender. Both parotids were tender, but not appreciably swollen, and there was pain on moving the jaw. The face was flushed, and the skin felt dry and hot. The temperature was 99.4 F., and the pulse was 84. On the 4th day the nasal discharge was still copious, frontal headache persisted, and he complained of aching pain in the back and extremities. The temperature was 99.4 F., and the pulse was 90. All symptoms ameliorated on the 5th day.

CASE 7.—The patient was not prone to respiratory infections, and had no cold during the winter. There was no history of recent exposure.

He was inoculated at 9 a. m., February 22, with 6 drops of nasal secretion Filtrate A in each nostril.

The onset was ushered in 26 hours after inoculation with dull frontal headache and attacks of sneezing. This was followed by a sensation of nasal stuffiness and dryness of the throat. In about 3 hours there was copious rhinorrhea and considerable lacrimation. During the evening he had slight tinnitus, and complained of deafness in both ears. He felt feverish, but did not record his temperature. He took 4 "rhinitis capsules" containing belladonna, and nasal secretion was perceptibly checked. He stated that he had several paroxysms of coughing during the night. The cough was dry, harassing, and unproductive. When seen the following morning, 48 hours after inoculation, he was coughing violently; there was a copious mucoid nasal discharge, and he was perspiring freely. The temperature was 99.4 F., and pulse was 88. These symptoms continued during the day and at 6.30 p. m., the temperature registered 100 F. On the 3rd day all symptoms ameliorated and the temperature was normal. Slight cough still persisted. On the 4th day he complained of slight sore throat, but felt well otherwise, and on the 5th day he was convalescent.

An interesting epidemiologic factor in this case is that during the 3rd day of his illness a niece living at his quarters became ill with an acute cold. It was apparently a contact infection.

CASE 8.—The patient gave a history of 2 mild attacks of coryza during the winter. There was no history of recent exposure.

He was inoculated at 9 a. m., February 22, with 6 drops of nasal secretion Filtrate A in each nostril.

Sneezing began about 6 hours after inoculation, and continued at intervals during the evening. There was the usual complaint of dull frontal headache. He slept well and on awakening the following morning his throat felt dry and parched, and nasal breathing was obviated by a sensation of stuffiness. A copious watery nasal discharge containing some mucus was noted during the forenoon, and he had to blow his nose repeatedly in order to breathe through it. Toward evening there were sensations of chilliness—"creeping chills," as he expressed it—but no distinct rigor. Sleep the 2nd night was disturbed by difficulty in breathing through nose. On the 3rd day he looked ill. The conjunctivae were injected, the eyelids were red and swollen, there was much lacrimation, and a copious rhinorrhea caused him to sniff and to use his handkerchief repeatedly, as he was interrogated. The upper lip and alae of the nose were excoriated. There was a slight unproductive cough. The nasal mucosa was very red, and showed marked boggy swelling. He felt listless and out of sorts. The temperature was 99.2 F., and the pulse was 120. Treatment was started and by the 5th day all symptoms had disappeared.

CASE 9.—The patient was prone to respiratory infections. He had adenoids. The tonsils were removed 8 months prior to inoculation. He gave a history of several colds during the winter.

He was inoculated at 9 a. m., February 22, with 5 drops of nasal secretion Filtrate C in each nostril.

The nose and throat became dry about 6 hours after inoculation. The following morning there was a slight nasal discharge which became blood-tinged after repeated blowing of the nose. He said he felt feverish during the day, but his temperature was not recorded. On the 3rd morning he complained of lassitude, and the nose and throat felt dry. There was moderate hoarseness. The temperature was normal. On the 4th day he complained of dull frontal headache, and on the 5th day there was slight sore throat and stiffness of the muscles of the neck. No elevation of temperature was noted.

This case is regarded as questionable because of the catarrhal diathesis of the subject and the reasonable doubt that arises as to whether the slight symptoms following inoculation indicate a true reaction or simply a slight flare-up of a chronic condition. The results lacked the clear cut and definite character of the other inoculation experiments.

CASE 10.—This soldier had several operations on the nasal septum prior to enlistment. The resulting scar tissue caused crust formation and difficulty in breathing. He stated that he was not prone to colds, and that he had not been ill during the winter. He was confined to hospital with a lumbar strain incurred in the gymnasium.

He was inoculated at 9 a. m., February 22, with 4 drops of nasal secretion Filtrate C in each nostril.

The soldier stated that he had several attacks of sneezing about 30 hours after inoculation, and that slight nasal discharge followed. On the 3rd day there was a sensation of fulness in the right ear, with slight impairment of hearing. He felt a little listless, and the nose felt dry. The temperature, recorded morning and evening, remained normal. The symptoms were slight and not clearly defined.

The reaction, if any, in this case was classified as doubtful. There was no febrile reaction, and anatomic defects existed that might readily account for the ill-defined symptoms recorded.

Analysis of the results of these experiments showed that of the 10 men inoculated, 7 developed clear cut and definite symptoms of acute coryza; 2 reacted questionably, while 1 remaining case exhibited no symptoms. There was an incubation period of 6 or 8-30 hours. The initial symptoms, as a rule, were dryness of the nose and throat and attacks of sneezing, dull frontal headache and a sensation of pain or fulness over the frontal sinuses. Several of the men complained of alternate sensations of chilliness and flushing. There was copious rhinorrhea, usually on the 2nd day, in a majority of the cases. Six of the subjects exhibited slight rise in temperature—99.2-100 F.—and in these cases the pulse was accelerated. Tinnitus aurium or slight impairment of hearing was recorded in 4 instances; sore throat in 5; cough in 5, and aching pains in the back or extremities

in 4. One of the men complained of parotid tenderness and pain on moving the jaw; in another case, a marked crop of herpes labialis was noted. The duration of the symptoms varied 3-6 days—usually 5.

During the period preceding these experiments, and while the observations were being made, there was no epidemic of colds in the command. The admission-rate for respiratory diseases was normal. The men were chosen at random as they volunteered and their duties, hygienic conditions, and general environment differed in no way from those of the remaining 250 men of the garrison. It would appear, therefore, that the experiments were adequately controlled.

CULTIVATION EXPERIMENTS

The results of the inoculation experiments with nasal secretion filtrates, and the sterility of these filtrates as regards micro-organisms capable of demonstration by ordinary bacteriologic methods, seemed to indicate clearly that the infective agent is a filtrable virus. The successful cultivation of the parasite of rabies by Noguchi,⁶ and the causative micro-organism of poliomyelitis by Flexner and Noguchi⁷—both organisms being “filter passers”—suggested the application of methods similar to those of Noguchi in attempting to grow an organism from the nasal secretion filtrates.

Several mediums were used in the earlier experiments and of these the most suitable has proved to be tissue-ascites fluid. About 12 c.c. of ascites fluid is poured into each of several test tubes measuring 1 × 20 cm., and a small piece of sterile fresh rabbit kidney is placed in the bottom of each tube with strict aseptic precautions. About 4 c.c. of sterile liquid petrolatum is then poured on the fluid medium and the tubes incubated for several days at 37 C., and at room temperature to insure their sterility. After a day or so, some of the blood from the kidney undergoes hemolysis and imparts to the medium surrounding the fragment of tissue a rose-colored tint; but in tubes that are not contaminated, this zone of hemolysis remains brilliantly clear. Any tubes showing haziness or cloudiness should be discarded.

After 18 months' experience with this technic, involving the examination of several hundred cultures prepared from many samples of ascites fluid and the tissue from many rabbits—not only in the work to be reported but in other studies—I am convinced that one should approach this work with the greatest patience and care, as successful results depend largely on the technical considerations involved. A visit to the Rockefeller Institute cannot help but impress one with the reasons for Noguchi's success in cultivating organisms that had previously proved refractory; and one should hesitate in attacking such work, because of his inability to confirm it, unless he has facilities equal to those of the original workers and considerable preliminary experience with the technic.

⁶ Jour. Exper. Med., 1913, 18, p. 314.

⁷ Ibid., p. 461.

The choice of a satisfactory ascites fluid is of prime importance. Certain workers have given standards by which the fluids may be judged, such as specific gravity, color, fibrin content, and the presence or absence of bile. In my work I have not depended on any of these considerations, as it is conceivable that a fluid that offers a satisfactory medium for some micro-organisms may not be suitable for the growth of others. Each attempt should be conducted with mediums made from several different ascites fluids, and when by actual test one is found that proves satisfactory, it should be carefully husbanded in the cold-room for future use. The ascites fluid should be originally sterile, and not subsequently sterilized by filtration or other methods.

Healthy rabbits should be chosen to furnish the tissue fragments used. It is my custom to strap the rabbit to an animal-board, cut the hair from the entire thorax and abdomen with scissors, and remove the remaining short hairs with a razor or a strong solution of sodium sulphite. The animal is stunned by a blow on the head, and a sharp bistoury is passed through the tissues of the neck so as to sever the carotid and jugular. The foot of the board is raised, and the body quickly becomes drained of blood. Death is instantaneous and painless. The entire abdomen is painted with a thick coating of tincture of iodine. The body of the animal and the board to which it is strapped are then enveloped in sterile towels saturated with a strong solution of liquor cresolis compound, leaving the portion of the abdomen that has been painted exposed, and taken to a dust-proof room where the remainder of the procedure is carried out. Gauze is tied over the nose and mouth of the operator and his assistant as a precautionary measure against contamination. All instruments used in the operation are sterilized by dry heat at 170 C., for at least 2 hours. They are brought to the operating room in the unopened pipet box in which they were sterilized. A median incision is made through the skin with a scalpel from the upper part of the sternum to the pubic region and, grasping the edges of the incised skin with rat-tooth forceps, it is dissected back and held out of the way with artery forceps. The instruments used thus far are now discarded. Next a spatula is heated to redness, and by repeated applications a semilunar sear is made on the exposed tissues, extending from the left inguinal region to the lower border of the ribs on the same side, its convexity being over the right side of the abdomen. The tissues underlying the lower part of the sear are picked up with forceps, and the abdomen is opened by cutting through the seared line in its entire extent. The resulting semilunar flap is turned back and held out of the way with a clamp. The intestine is now lifted out of the way and a kidney exposed. With fresh forceps the kidney is quickly lifted from its bed and transferred to a large sterile culture dish. The capsule is stripped from the kidney with forceps and the organ is then cut into 12-15 pieces with small sterile scissors. The assistant holds the cover of the dish over the field during this procedure. The remaining kidney is disposed of, of course, in the same manner. The fragments of tissue are then transferred to the culture tubes with long slender forceps or, if these are not at hand, by picking them up on a hot platinum loop to which they adhere. The test tubes are sterilized by dry heat and the necks are flamed just before and just after adding the ascites fluid and the tissue. The liquid petrolatum that is superimposed on the medium is sterilized in the autoclave under 15 pounds pressure, on 2 successive days.

Rigid adherence to this technic results in exceedingly few contaminations. Occasionally, after incubation, a sample of ascites fluid shows contamination with a small gram-positive coccus; but all the tubes will show the same organism, and one cannot be misled if each culture is controlled by tubes of unin-

oculated medium incubated with the cultures. Another occasional source of contamination is a small pleomorphic diphtheroid organism that apparently comes from the tissue. In each experiment the sterility of the medium should be insured by incubation prior to inoculation, and the procedure further safeguarded by incubating control tubes of uninoculated medium with the inoculated tubes.

Sterile capillary pipets, with the capillary end drawn out to a length of about 25 cm., are used in inoculating the tubes. The pipets are plugged with cotton, placed in large glass tubes plugged with cotton, and sterilized at 160 C., for at least 2 hours. The pipets are removed with care, the capillary end is flamed, and a rubber nipple is fitted to the proximal end. About 0.5 c.c. of the nasal secretion filtrate is drawn into the pipet and, being careful that no air bubbles are introduced below the layer of oil, the tip of the pipet is thrust through the medium to the bottom of the tube, and the filtrate introduced into that part of the medium surrounding the tissue. The introduction of air bubbles at the end of the procedure may be prevented by not entirely emptying the pipet of its contents. Originally, the cultures prepared in this way were incubated in an anaerobic jar. Later experience has shown that this is unnecessary as an adequate degree of anaerobiosis is occasioned by the fragment of tissue in the medium. In making initial cultures it is well, however, to incubate one series of cultures in an anaerobic jar and a duplicate series outside the jar.

MACROSCOPIC APPEARANCE OF CULTURES

An initial culture has now been obtained in 11 cases of acute coryza acquired in the usual way, and from 5 cases induced by experimental inoculation. The initial cultivation is attended with more or less difficulty; but once the growth has been established the virus tends to become saprophytic, and subsequent cultivation is attended with less difficulty. I have not succeeded in obtaining an initial growth in solid mediums. Subculture in solid mediums is possible, however, after growth for 2 or 3 generations in the fluid medium.

The appearance of initial cultures in tissue-ascites medium varies in degree with the various filtrates used as inocula, but is otherwise always the same. A distinct, filmy, grayish white, opalescent halo, superimposed on a cloudy hemoglobin-stained zone surrounding the tissue, is observed at the end of 24 hours. This halo or ring is sharply demarcated from the clear fluid above, and shows no tendency to blend with it. The appearances are not unlike those resulting from the addition of nitric acid to urine containing a trace of albumin. It is probable that this is partly due to chemical changes of a protein nature in the ascites fluid, induced by the virus or constituents of the secretion filtrate. At the end of 48 hours the changes are at least 100 per cent. more marked, while on the 3rd day the tissue is more or less obscured by the density of the cloudiness surrounding it. On the 4th day the upper column of fluid, which has heretofore remained clear,

shows slight uniform haziness which increases in density as time goes on. In old cultures the upper column of fluid becomes clear again, and a finely granular or powdery sediment collects around the tissue in the bottom of the tube.

Control tubes inoculated with salt solution show a very faint halo at the end of 24 hours, similar to that observed in the culture tubes; but there is no cloudiness in the zone surrounding the tissue, and the change does not become intensified on subsequent days. After 2 weeks' incubation some autolysis of the tissue becomes evident, and there is slight haziness of the supernatant fluid due to admixture of minute particles of autolyzed tissue.

The changes in tissue-ascites subcultures are more marked than in the initial cultures, and the cloudiness in the tissue zone exhibits more tendency to extend upward and diffuse into the clear medium.

In making subcultures in the solid medium, 0.5 c.c. of the Berkefeld filtrate of the subculture to be transferred is added to a sterile tube containing a piece of sterile rabbit kidney. A mixture of equal parts of ascites fluid and 2 per cent. nutrient agar is then added, and 3 or 4 c.c. of liquid petrolatum are superimposed on this. The agar is boiled to drive off the oxygen, and then rapidly cooled to 42 C. The ascites fluid is brought to the same temperature as the agar before admixture with it. The appearances of growth in this medium are similar in a way to those in the fluid medium. The growth appears in 4 or 5 days as a faint grayish film surrounding the tissue. Individual colonies are not discernible at this stage, even with the aid of a lens. The growth gradually extends upward and diffuses into the clear medium for a distance of 4 or 5 cm., the upper limits of the haziness blending imperceptibly with the clear medium. There is no tendency toward demarcation of the growth, such as occurs in the fluid medium. After several days' growth extremely minute colonies that may be brought out with a lens are demonstrable in the zone immediately above the tissue. These colonies occur only in this limited zone, and here the growth is most dense. The growth in the solid medium is much slower than in the tissue-ascites medium, and I have been able to obtain the growth only in subcultures after cultivation for 2 or 3 generations in the fluid medium.

DEMONSTRATION OF A MICRO-ORGANISM IN CULTURES

In my preliminary communication⁵ I stated that stained preparations from the cultures showed no bodies that could be "definitely recognized" as micro-organisms, although "minute coccoid bodies and occasional rodlike forms were demonstrable in all the preparations, but not in sufficient numbers to preclude the possibility that they were artefacts." The original cultures were examined on the 3rd or 4th day, and only the uppermost limits of the macroscopic growth was examined. Shortly after submitting this report for publication addi-

tional preparations were studied from cultures that had been incubated 14 days, the material pipetted from the bottom of the tubes in the zone surrounding the tissue. Innumerable coccoid bodies were demonstrable in these preparations.

Subsequent experience has shown that the original failure to demonstrate the micro-organism in unmistakable numbers was due to the preparations having been made from early cultures, before appreciable multiplication had occurred, and to the selection of a zone of the cultures where, at best, growth is scant. This oversight was occasioned by a deliberate effort to avoid the zone surrounding the tissue, as it was thought that products of tissue autolysis might give rise to confusing artefacts.

Stained preparations from cultures that have been incubated 7-14 days—the period during which maximum growth occurs—show extremely minute coccoid bodies occurring singly, in pairs, and in agglomerations of varying size. Occasionally there appears to be slight tendency to chain-formation. The chains are not numerous and they are short, consisting of 4-6 elements. The bodies show marked variation in size, but are uniformly coccoid or globular in shape. Forms just approaching visibility, bodies larger than a staphylococcus and intermediate forms exhibiting all gradations in size are demonstrable frequently in a single group. The larger forms often show minute bodies adherent to them, which suggests that multiplication occurs by budding. The predominating type is uniformly the small globoid form measuring 0.2-0.3 of a micron in diameter.

The micro-organism stains well with Giemsa's stain, and less clearly with the ordinary laboratory stains. The bodies in young cultures are gram-negative. In old cultures some of the bodies, especially the larger forms, are variable in their behavior toward the gram stain, retaining the gentian violet, or only becoming decolorized after repeated washing with alcohol.

The most satisfactory results have been obtained with Giemsa's stain. The preparations are air-dried without heating, fixed in absolute methyl alcohol for 1 hour, washed in water, and then stained over night in a Coplin jar containing Giemsa's solution in the proportion of 1 large drop of the stain to each cubic centimeter of distilled water. To avoid artefacts any excess of stain is removed by immersing the preparation in acetone for a few seconds, and then washing thoroughly in distilled water. A majority of the bodies are stained a distinct purple by this method. In old cultures, however, there is more or less variability in the color, some staining deep blue and others reddish. Varying degrees of intensity of staining are also evident in old cultures, the extremely minute, and very large forms staining faintly, while the intermediate forms are colored a deep blue. Some of the bodies stain poorly and appear to be inclosed in a more deeply staining peripheral structure. It is not certain whether this indicates the possession of a true capsule or a 'pseudo-capsule' composed of albuminous material from the culture medium. Most of the tinctorial and morphologic characteristics are evident in the photomicrographs (Figs. 2, 3, and 4.)

It becomes evident at once that this micro-organism differs markedly from any known organism with the possible exception of the

"globoid bodies" described by Flexner and Noguchi,⁷ and believed by them to be the causative factor in poliomyelitis. An extremely pleomorphic streptococcus, the minute forms of which bear some resemblance to this micro-organism, has recently been described in connection with poliomyelitis, also, by Mathers,⁸ Rosenow, Towne and Wheeler,⁹ and Nuzum and Herzog.¹⁰

Through the courtesy of Dr. Noguchi and Dr. Rosenow, I have been able to study preparations of the micro-organisms described by them and to compare these with my own preparations. The slide received from Dr. Rosenow shows an extremely pleomorphic coccus ranging in size from elements that just approach visibility to forms fully as large as a pneumococcus. The arrangement is in clumps, as a rule, with a very definite tendency to chain-formation. The pleomorphism observed in this preparation is striking. The predominating form is coccoid in shape, stains with moderate intensity, and is arranged in groups and chains. A moderate number of large cocco-bacilloid forms are observed, occurring singly, in pairs, and in short chains of up to 8 elements. These forms have a lightly stained zone across the lesser diameter and the ends stain deeply, giving them the appearance of coccobacilli. Numerous bacilloid types also occur. Of these some exhibit polar staining, others appear fusiform, and bizarre types with clubbed ends and sporelike bodies also occur. Dr. Noguchi's preparation shows small globoid bodies that are uniform in size and shape. These bodies are extremely minute, measuring 0.2-0.3 of a micron in diameter. They occur singly, in pairs, in short chains, and in groups. Occasionally the more deeply stained elements appear to be larger than the remainder, but there is no marked variation in size. In common with these 2 poliomyelitis types, the micro-organism isolated in common colds shows as its predominating type minute coccoid or globoid bodies arranged in groups. Unlike the Flexner-Noguchi bodies, however, there is considerable variation in the size of the bodies, and some of the larger ones show possible budding. While the coccoid bodies resemble the smaller forms observed in Dr. Rosenow's preparation, there is no approach to the pleomorphism seen in the latter—variations in the cold organism being limited to differences in size—and cocco-bacilloid forms do not occur. Furthermore, there is not the tendency to streptococcus types that is so characteristic of the Rosenow organism.

The cultural requirements of the micro-organism isolated in common colds are similar to those of the globoid bodies of poliomyelitis, initial cultivation being obtained with difficulty, and only in the selective Noguchi medium; growth is slow—the micro-organism not being demonstrable in numbers before the 6th or 7th day—and never luxuriant; and cultivation in solid mediums is not possible until the organism has become more or less saprophytic by growth for several generations in the fluid medium. Again, cultivation has not been possible under aerobic conditions. On the other hand, the streptococcus

⁸ Jour. Am. Med. Assn., 1916, 67, p. 1019.

⁹ Jour. Am. Med. Assn., 1916, 67, p. 1202.

¹⁰ Jour. Am. Med. Assn., 1916, 67, p. 1205.

described by Rosenow and his co-workers is cultivated with comparative facility, and growth is luxuriant on a variety of mediums under both aerobic and anaerobic conditions, but always best under aerobic conditions.

FILTRABILITY AND INFECTIVITY OF CULTURES

The inoculation experiments with nasal-secretion filtrates, previously cited, indicate that the virus of common colds is filtrable, and the interesting questions now arise as to whether the virus is capable of being cultivated *in vitro*; whether the appearances of macroscopic growth observed in cultures are in reality due to the multiplication of this virus; and, finally, whether the minute micro-organism that has been described bears any relation to the true infective agent.

Experimental evidence has been obtained that should leave little doubt that the virus is capable of cultivation, and that the virus so cultivated is infective after passage through a Berkefeld N filter. Additional experiments strongly suggest, although conclusive proof has not been obtained, that the minute micro-organism demonstrated in cultures is the true infective agent. This micro-organism has been demonstrated in subsequent generations of subcultures that had proved infective by inoculation experiments; and it has been proved that the micro-organism is filtrable by the passage of fluid medium cultures through Berkefeld N filters and the subsequent demonstration of the micro-organism in both fluid and solid mediums inoculated with the culture filtrates.

The inoculation experiments that follow, however, were made early in the investigation—during March, 1916—and, due to the technical oversight that has been mentioned already—examining the cultures too early and selecting a zone where growth is scant—micro-organisms in unmistakable numbers were not demonstrable. In subsequent subculture generations of the 2 viruses used, however, the minute micro-organism was demonstrated unmistakably.

Subcultures of the nasal secretion filtrate Viruses A and B, that had proved infective before cultivation in the first series of inoculation experiments, were used in the experiments that follow. These subcultures were of the 2nd generation. Portions were diluted 10 times with sterile normal salt solution and passed through Berkefeld N filters. The resulting filtrates were perfectly clear and proved sterile as regards organisms capable of growth on aerobic and anaerobic blood-agar plates.

By dilution of the nasal secretion with salt solution, the further dilution occasioned by the fluid-culture mediums, and the final dilution of the portions of subcultures used with sterile salt solution, the constituents of the original nasal secretions that were capable of filtration had reached, in these filtrates, a dilution of 1:90,000 plus the unknown original dilution occasioned by the fluids of the nasal secretion itself.

Eleven soldiers voluntarily submitted to inoculation with these subculture filtrates. The technic of inoculation was identical with that used previously in the experiments with nasal secretion filtrates. Five drops of the diluted culture-filtrate were dropped into each nostril from a sterile capillary pipet.

CASE 1.—The patient was not prone to colds, although he usually had 1 or 2 attacks during the winter. He had not had a severe cold during the present season.

He was inoculated at 8:30 a. m., March 3, with 5 drops of diluted subculture-filtrate of Virus A in each nostril. The filtrate was 18 hours old.

At 2:30 p. m., 6 hours after inoculation, he experienced chilly sensations, followed by unilateral nasal stuffiness and a sensation of fulness over the frontal sinuses. The temperature at 5 p. m., was 99 F., and the pulse was 80. The following day the nasal mucosa was swollen and boggy. There was slight nasal discharge. The only subjective symptoms were nasal stuffiness and attacks of sneezing. The temperature was 98.2 F., and the pulse was 72. On the 3rd day all symptoms had ameliorated.

CASE 2.—The patient very seldom had a cold. He had not been ill during the winter.

He was inoculated at 8:30 a. m., March 3, with 5 drops of diluted subculture-filtrate of Virus A in each nostril.

The soldier stated that he developed severe frontal headache and dryness of the nose and throat about 8 hours after inoculation. There was a sensation that the nose was running, but no secretion was evident. At 5 p. m., he complained of difficulty in breathing through the nose, and examination showed the mucosa to be much swollen. The temperature was 98.8 F. The patient was not seen again, and there is no record of subsequent symptoms.

CASE 3.—The patient stated that he was moderately susceptible to infection. There was history of an acute cold 2 months prior to inoculation.

He was inoculated at 8:30 a. m., March 3, with 5 drops of diluted subculture filtrate of Virus A in each nostril.

When seen at 5 p. m., 8 hours after inoculation, he stated that he had had several paroxysms of sneezing, and he complained of slight unproductive cough. Moderate rhinorrhea was evident, and the nasal mucosa was much swollen and very red. The temperature was 99 F., and the pulse was 76. The following day the nasal secretion was more copious. On the 4th day all symptoms had disappeared.

CASE 4.—The patient was not susceptible to colds.

He was inoculated at 8:30 a. m., March 3, with 5 drops of diluted subculture filtrate of Virus A in each nostril.

At 5 p. m., on the day of inoculation he had slight frontal headache. There was also complaint of dryness of the throat, and a burning sensation in the nose when breathing. The mucosa was swollen, and there was slight nasal discharge. Twelve hours after inoculation he felt feverish, but the temperature was not recorded. The day following inoculation there was complaint of nasal stuffiness, he was hoarse, and there was a slight cough. The nasal mucosa was much congested, swollen, and moderate rhinorrhea was evident. The temperature was 98 F. Later in the day he had several attacks of sneezing. On the 3rd day there was copious rhinorrhea. Symptoms began to ameliorate on the 4th day, and had entirely disappeared by the 6th day.

CASE 5.—The patient recovered from an acute cold a week prior to inoculation. He was selected for inoculation to test immunity.

He was inoculated at 10 a. m., March 3, with 5 drops of diluted subculture filtrate of Virus A in each nostril.

The soldier stated that during the evening of the day of inoculation there was slight headache. On the 2nd day the headache still persisted, and there were several attacks of sneezing. During the evening of the 2nd day there was copious rhinorrhea, the conjunctivae were injected, and there was considerable lacrimation. The temperature was normal. Symptoms continued another day, and then rapidly ameliorated.

CASE 6.—The patient stated that he is not susceptible to infection. He had not had a cold for several months.

He was inoculated at 10 a. m., March 3, with 5 drops of diluted subculture filtrate of Virus B in each nostril. He returned to Fort Andrews after the inoculation and notes on the course of subsequent events were furnished by Sergeant 1st Class Crampton, Hospital Corps.

Twenty-four hours after inoculation the mucous membrane of the nose was red and swollen, and the patient was breathing through his mouth. He complained of nasal stuffiness and slight frontal headache. The headache ceased about 2:30 p. m., and its cessation was followed by the appearance of a thin, clear, nasal discharge. The temperature was 99.2 F. The following day the nasal secretion had diminished, and had ceased by evening.

CASE 7.—The patient stated that he had a cold a month prior to inoculation, but that he was not susceptible as a rule.

He was inoculated at 10 a. m., March 3, with 5 drops of diluted subculture filtrate of Virus B in each nostril. He was returned to his station and subsequent events were noted by the post surgeon.

A nasal discharge was noticed about 8 hours after inoculation with attacks of sneezing and coughing. The temperature remained normal. The nose felt "stopped up" and he complained of a burning sensation over the eyes. The following morning he still complained of nasal stuffiness, and there was profuse, thin, nasal discharge and considerable cough. The temperature was 99 F. On the 3rd day there was slight difficulty in breathing through the nose, and slight cough persisted. On the 4th day he was well.

CASE 8.—The patient stated that he had not had a cold for years.

He was inoculated at 10 a. m., March 3, with 5 drops of diluted subculture filtrate of Virus B in each nostril. He returned to his station after inoculation and recorded his own symptoms.

On the evening of the day of inoculation he felt "as if he were taking a cold." There was a slight mucoid nasal discharge, and the throat felt dry. No rise in temperature was recorded. An abortive attack occurred.

CASE 9.—The patient recovered from an experimental cold induced with nasal secretion filtrate a few days prior to inoculation. He was inoculated again to test immunity.

He was inoculated at 10 a. m., March 3, with 5 drops of diluted subculture filtrate of Virus B in each nostril.

During the afternoon he had several attacks of sneezing, and toward evening dull frontal headache, with a sensation of nasal stuffiness, developed. The temperature was normal. The following day there was copious rhinorrhea and cough. The temperature was still normal. The 3rd day all symptoms had disappeared.

CASE 10.—This soldier had been inoculated, also, with nasal-secretion filtrate prior to this experiment.

He was inoculated at 10 a. m., March 3, with 5 drops of diluted subculture filtrate of Virus B in each nostril.

Eight hours after inoculation he complained of dull pain over the eyes, and the neck felt stiff. The temperature was 99.4 F., and the pulse was 84. The following day he complained of dizziness, dull frontal headache, and aching pains in the back and extremities. The nose and throat felt dry and parched. The temperature was 97.6 F., and the pulse was 72. The symptoms gradually ameliorated and by the 5th day he felt well.

CASE 11.—The patient had recently recovered from a severe cold induced by experimental inoculation with nasal-secretion filtrate.

He was inoculated at 10 a. m., March 3, with 5 drops of diluted subculture filtrate of Virus B in each nostril.

During the afternoon of the day of inoculation he had 2 attacks of sneezing; there was slight rhinorrhea and dull frontal headache. At 5 p. m., the temperature was 99.4 F., and the pulse was 80. The following day he felt out of sorts, complained of dull aching pains in the back and extremities, his throat was dry and sore, there was a slight mucoid nasal discharge, and the afternoon temperature was 99.2 F., and the pulse was 96. The 3rd day the symptoms were the same as on the 2nd. On the 4th day the nasal discharge was profuse, and the alae of the nose and upper lip had become excoriated. He sneezed frequently, the eyelids were red and puffy, and he "looked ill." The temperature at this time was 99 F. The symptoms gradually ameliorated from this time, and by the 7th day he had entirely recovered.

These experiments show that, after incubation periods of approximately the same duration as those occurring in the earlier experiments with nasal-secretion filtrates, unmistakable symptoms of acute coryza were exhibited by the subjects. In one case the attack was mild or abortive, but in the remaining 10 cases the symptoms were well-defined and persisted for several days. The results in Cases 5, 9, 10, and 11, although in accord with clinical and epidemiologic knowledge of the disease, are interesting in their bearing on the questions of immunity and specific therapy. The patient in Case 5 had recently recovered from an acute cold acquired naturally, and the patients in Cases 9, 10, and 11 had recovered but a few days previously from colds induced by experimental inoculation; yet, all of these subjects proved sus-

ceptible to inoculation with the subculture-filtrates. These results suggest that immunity of even short duration is not conferred in this condition, and that specific vaccine therapy would be of questionable value, even were the virus readily cultivable.

Berkefeld filtrates were prepared from the nasal secretions in 5 of these experimental cases, 48 hours after inoculation. Tubes of tissue-ascites medium were inoculated with 0.5 c.c. each of the filtrates, in the manner previously described. Four of the cultures showed the characteristic changes observed in previous cultural experiments. Cultures in the 5th case remained unchanged at the end of 24 hours, but another nasal secretion filtrate from this case, collected 72 hours after inoculation, gave positive results.

The minute micro-organism that has been described was not demonstrated in the initial cultures from these cases. It was demonstrated subsequently, however, in the 3rd subculture generation of the virus from one of the patients (Case 11). The micro-organism has been demonstrated, also, in subcultures of Viruses A and B that were used in both series of inoculation experiments, and in the initial cultures from the nasal secretion filtrates of 7 cases. It has never been encountered in control tubes of uninoculated medium which were uniformly incubated with each series of cultures.

DISCUSSION

The experimental evidence that has been presented appears to show that common colds are infectious; that the causative virus occurs in the nasal secretions, and that this virus is capable of passing through Berkefeld N filters that are impermeable to ordinary bacteria. While many of the known organisms that have been described in connection with the causation of common colds, such as the pneumococcus and various streptococci, may act as secondary invaders, prolonging or intensifying the attack or acting as the true cause of numerous complications or sequelae, it is believed that the evidence supports the probability that the primary infective agent is the filtrable virus that has been described.

The question arises as to whether the symptoms following inoculation with this virus might not be explained by the action of a preformed toxin or enzyme in the nasal secretions. Such an explanation is believed to be untenable in view of the distinct latent period that uniformly elapsed between inoculation and the onset of symptoms, together

with the occurrence of slight fever in a majority of the cases. As far as is known, inoculation with toxin is followed by almost immediate reaction, and the symptoms are transitory and never prolonged. The existence of this silent or latent incubatory period also serves to dismiss anaphylactic phenomena from consideration.

The cultural experiments suggest that a living virus has been cultivated *in vitro*, and that the virus so cultivated is infective in at least the first subculture generation. The filtrable constituents of the original nasal secretions were in a dilution of 1:90,000+ in the filtrates of subcultures which produced colds experimentally; and it is inconceivable that any toxin, virus, or enzyme, preformed in the secretions, could have been carried over mechanically and, in this high dilution, exhibited pathogenicity. Such pathogenicity is common only to living bodies capable of growth and multiplication. Secondly, the cultures showed changes that ordinarily are interpreted as indicative of micro-organismal growth, while identical changes were not observed in control tubes. Again, the changes observed in subcultures were intensified in each successive subculture generation, thus bearing an inverse relation to the dilution of the inoculum originally introduced. This phenomenon frequently occurs during the continuous cultivation of known organisms, as the result of acquired adaptability to the conditions in an artificial medium. Finally, minute coccoid bodies have been demonstrated in initial cultures from the filtered nasal secretions of persons ill with acute colds; in subsequent generations of subcultures that had proved infective by inoculation experiments; and in subsequent generations of a culture from the filtered nasal secretion of a person with the symptoms of an acute cold induced experimentally. These bodies have never been encountered as ordinary saprophytes, nor have they been demonstrated in control tubes of uninoculated medium incubated with each series of cultures. The bodies are capable of passing through Berkefeld N filters, as is shown by demonstrating the bodies in cultures and then recultivating identical bodies from the culture filtrates. Because of the extreme minuteness and, at times, indeterminate morphology of many of the bodies, doubt may arise as to their true micro-organismal nature. Any such doubt should be dispelled by the fact that the bodies finally have been grown in a solid medium which obviates the entrance of artefacts that might occur in fluid mediums.

In the absence of conclusive experimental proof, interesting theories suggest themselves as to the relation of the minute micro-organism

described to the true infective agent. It is conceivable that this visible micro-organism might co-exist in cultures with an ultramicroscopic virus, the latter being the true infective agent; but, as pointed out by Flexner and Noguchi¹⁷ in discussing this same question as applied to the micro-organism causing poliomyelitis, an instance of symbiosis of this nature is not known to animal pathology. On the other hand, recent studies in cerebrospinal meningitis, conducted by the English investigators, Hort, Lakin, and Benians,¹¹ raise the question whether certain micro-organisms do not exhibit a complex life cycle somewhat similar to that which obtains among the protozoa and which appears to obtain among the spirochetes. These workers believe that the meningococcus as it occurs in the cerebrospinal fluid and in cultures is not the true causative factor in epidemic cerebrospinal meningitis. The results of their cleverly conceived and carefully executed experiments suggest that the true infective agent is a filtrable virus and that the meningococcus, as we know it, although not a genuine secondary invader, probably represents a late phase in the life history of an unidentified minute micro-organism that is the true infective agent. In support of this theory they showed that it was possible, under favorable conditions, to demonstrate in the cerebrospinal fluid of acute human cases of epidemic meningitis the presence of a pathogenic virus capable of passage through Chamberland F bougies. The filtrates when injected intraperitoneally into monkeys caused continued fever and death. There was a definite incubation period which should preclude the possibility that the febrile reaction was induced by preformed pyrogenic toxins. As opposed to this, they showed that the injection of other monkeys with cerebrospinal fluid taken from acute cases of the disease produced no effect, notwithstanding the fact that living meningococci were cultivated in numbers from the fluid injected.

In a subsequent paper Hort and Caulfeild¹² report the results of further experiments and conclude that the pathogenicity to monkeys of cerebrospinal fluid from acute cases of cerebrospinal fever appears to tend to vary inversely as the meningococcal content; that the cerebrospinal fluid in this disease contains a filter-passing agent that is not the meningococcus, but which is capable of producing in monkeys continued fever and death; that the filtrable agent is a living virus capable of cultivation and of passage through monkeys, and that the patho-

¹¹ Jour. Roy. Army Med. Corps, 1916, 26, No. 2.

¹² Ibid., 27, p. 312.

genicity of the meningococcus, as such, appears to be due to the concomitant presence of the filter-passing virus described.

If one reverts to the description that have been given of the morphology and cultural characteristics of the micro-organism isolated in common colds, it is evident that certain facts lend themselves to serious consideration in connection with the theory advanced by Hort and his co-workers. In the first place, the cultures show changes indicative of macroscopic growth several days before visible micro-organisms are demonstrable in numbers, the latter only appearing after cultures have been incubated for 7-14 days. Secondly, the micro-organisms that are demonstrable at first are extremely minute and scant in numbers. Later, as multiplication occurs, larger forms appear, many of which seem to be budding, while others appear to be inclosed in a peripheral structure. Finally, in the older cultures, the micro-organisms show all gradations of size from bodies that are just visible under a high power lens to bodies larger than a staphylococcus.

Is it not conceivable that the gradations of size do not stop with the bodies that are just visible, but that myriads of still more minute forms may occur, causing the early cloudiness in cultures that is out of proportion to the number of demonstrable micro-organisms; and that these possible ultramicroscopic forms represent an early phase of a complex life cycle during which the micro-organism is infective, while the larger bodies, which are visible under the microscope, represent a resting stage in the life history?

Another question that arises is whether the coccoid bodies may not represent mutations of one of the more commonly known organisms. Dr. E. C. Rosenow has informed me that pneumococci placed under cultural conditions similar to those governing the growth of the micro-organism isolated in common colds have shown pictures similar to those seen in preparations of the minute micro-organism described, particularly after filtration of cultures which have shown every gradation from just visible bodies to large diplococcus forms. In view of the uniformity with which pneumococci occur in the mouth and throat, and their frequent presence in the secretions in the later stages of common colds, it is possible that there may be some relation between the bodies described and the pneumococcus. On the other hand, these bodies never become aerobic, even after repeated cultivation; the employment of a special method is required for their cultivation, and

growth does not occur in mediums that furnish pabulum for the pneumococcus. These facts militate against the assumption that there may be a relationship between the micro-organisms.

No opinion is hazarded as to the classification of the peculiar micro-organism that has been described. Moreover, a definite statement at this time in regard to its real relation to the causative agent of common colds would, in the absence of further proof, be based on questionable premises. The fact remains that a very interesting micro-organism has been isolated from the filtered nasal secretions of persons ill with common colds, and sufficient evidence that it bears some relation to the true infective agent has been adduced to merit further investigation.

CONCLUSIONS

From the experimental evidence presented it seems that the following facts have been established:

Common colds of the ordinary type are infectious.

The ordinary bacteriologic methods that have been resorted to, heretofore, do not furnish reliable criteria on which to base conclusions as to the etiology of these affections. Cultures made from the nasal secretions early in the acute phase often remain sterile, while cultures made later in the attack frequently show such a diversity of organisms that only presumptive evidence exists for ascribing to any one an etiologic rôle.

It has been demonstrated experimentally that the virus of common colds occurs in the nasal secretions; and that this virus is capable of passing through Berkefeld filters which are impermeable to ordinary bacteria.

By the employment of special anaerobic methods the virus of common colds has been cultivated *in vitro*, and has proved capable of repeated recultivation in subcultures.

Experimental inoculations have demonstrated that Berkefeld N filtrates of subcultures of the virus, in the 2nd generation at least, are infective.

A peculiar minute micro-organism has been isolated from cultures made from the filtered nasal secretions in common colds. This micro-organism can be passed through Berkefeld N filters, and has been recultivated from culture-filtrates. Although conclusive proof of its nature has not been adduced, the experiments suggest that the micro-organism described bears a definite relation to the true infective agent.

PLATE 9

FIG. 1. Macroscopic appearances of tissue-ascites medium cultures of the virus of common colds. Cultures incubated 7 days. The tube to the extreme left is a control tube that was incubated with the cultures.

FIG. 2. Appearances of the minute coccoid bodies isolated from a 14-day culture. Note the variation in size and the intensity with which the stain is taken. Giemsa stain. $\times 1000$.

FIG. 3. Large groups of the coccoid bodies in a 14-day culture. Giemsa stain. $\times 100$.

FIG. 4. Selected fields seen in culture preparations of the micro-organism isolated in common colds. Note the occurrence of single bodies and the arrangement in pairs, short chains, and small groups. The upper right field illustrates the appearance of the large budding forms that have been described as occurring in old cultures. The lower right field shows a group of bodies that appear to be inclosed in a peripheral structure. Giemsa stain. $\times 1000$.

PLATE 9

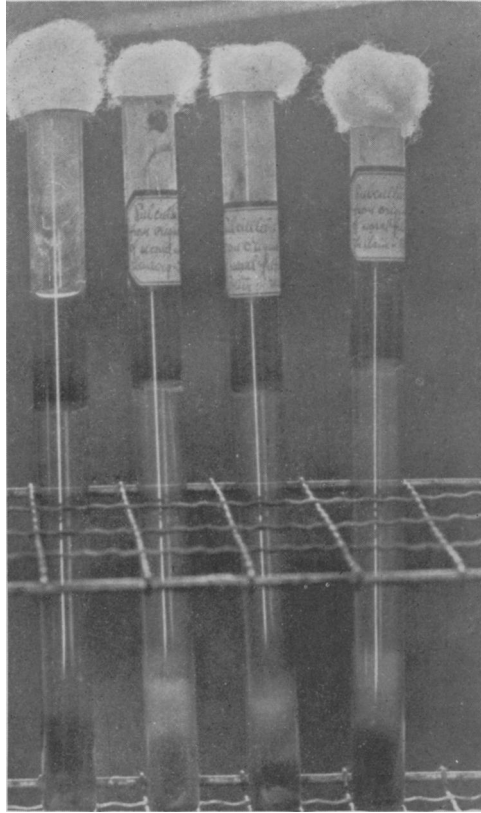


Fig. 1.

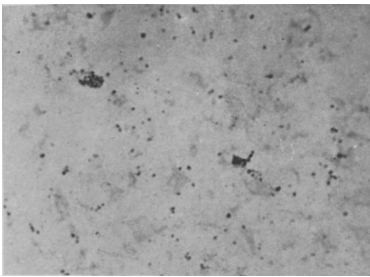


Fig. 2.

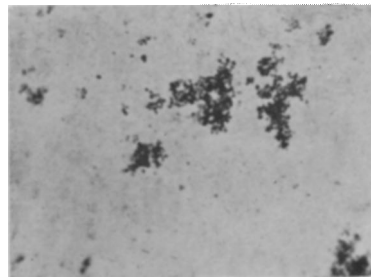


Fig. 3.

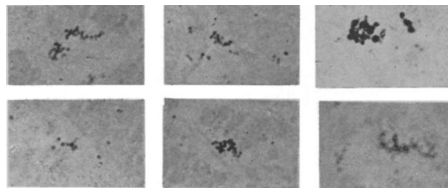


Fig. 4.